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RELATIVE TOXICITY OF CHLOROPICRIN, PHOSPHINE, EDC-CCl₄, AND CCl₄-CS₂ TO VARIOUS LIFE STAGES OF THE INDIAN MEAL MOTH

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CONTENTS

	Page
Summary	. 1
Background and objectives	. 1
Materials and methods	. 2
Results	. 2
Chloropicrin fumigations	. 2
Phosphine fumigations	. 3
EDC-CCl ₄ fumigations	. 4
CCl ₄ -CS ₂ (80:20) fumigations	. 5
Untreated control test insects	. 5
Discussion	. 6
Literature cited	8

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RELATIVE TOXICITY OF CHLOROPICRIN, PHOSPHINE, EDC-CCl₄, AND CCl₄-CS₂ TO VARIOUS LIFE STAGES OF THE INDIAN MEAL MOTH

By C. L. Storey and L. I. Davidson¹

SUMMARY

Eggs, larvae, and pupae of the Indian meal moth, Plodia interpunctella (Hübner), were exposed to various concentrations of four fumigants for exposure periods ranging from 4 to 72 hours. The order of tolerance to phosphine, $EDC-CCl_4$ mixtures, and CCl_4-CS_2 mixture was eggs > pupae > 5-week-old larvae > 2-week-old larvae. The order of tolerance to chloropicrin was pupae > eggs > 5-week-old larvae.

Although pupae were slightly more resistant than eggs to treatment by chloropicrin, adults emerging from pupae that survived concentrations giving complete control for eggs failed to produce a second generation. Eggs were particularly resistant to phosphine, surviving 24-hour exposures of 0.70 milligrams per liter (mg./l.) (500 p.p.m.) and 72-hour exposures of 0.083 mg./l. (60 p.p.m.). Chloropicrin prevented hatching of the eggs at concentrations of 2 mg./l. in 24-hour exposures and 0.5 mg./l. in 48-hour exposures. Some difference in toxicity was found between EDC-CCl₄ mixtures in ratios of three parts EDC to one part CCl₄ and one part EDC to one part CCl₄. Each ratio was effective at total component concentrations as low as 3 mg./l. in 24 hour exposures; however, partial development in the pupal and egg stages was observed in the one to one ratio fumigations. The 80:20 mixture of CCl₄-CS₂ was the least effective of the fumigants tested. No CCl₄-CS₂ fumigation with a concentration multiplied by time giving a product below 1200 (50 mg./l. \times 24 hours or 25 mg./l. \times 48 hours) gave complete control of the life stages tested.

BACKGROUND AND OBJECTIVES

The Indian meal moth, *Plodia* interpunctella (Hübner), is recognized as a serious pest of stored products that may appear in tremendous numbers wherever grain is stored (1)². Recommendations for control of this moth include fumigation and the use of surface sprays, pyrethrins-piperonyl butoxide (6) and malathion (11). During recent years, malathion has generally replaced the pyrethrins-piperonyl butoxide treatments which were expensive and required frequent applications to be effective. Numerous reports, however, have been received concerning the developing resistance of Indian meal moth to malathion treatments. La Hue (2) reported that progeny from a field resistant strain survived laboratory applications of 20 and 40 p.p.m. of malathion in corn. This situation has resulted in renewed interest in the use of fumigants for moth control. The study reported here was conducted to determine the relative toxicity of some of the commonly used fumigants to the various life stages of the Indian meal moth.

² Italic numbers in parentheses refer to Literature Cited, Page 8.

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MATERIALS AND METHODS

Fumigations were conducted in 19.5 liter glass bottles sealed with plastic caps. A small rubber serum stopper was inserted through each cap to permit use of a microsyringe for sampling of the gas concentrations within the bottle. A column of four interconnected perforated polystyrene specimen bottles small enough to pass through the narrow neck of the glass bottle served as test cages. The cages and a remote humidity sensor were suspended beneath the glass bottle cap. Life stages of the Indian meal moth placed in the four cages included fewer than 100 eggs, 0 to 24 hours old, 10 larvae 2 weeks old, 10 larvae 5 weeks old, and 10 pupae. A small amount of suitable food material was placed in each cage.

Following exposure periods ranging from 4 to 72 hours, the test insects were placed in small plastic boxes containing food material, and they were stored at 80° F. and 60 percent relative humidity until post-fumigation mortality and development observations were completed.

During the post-fumigation period, the eggs were examined initially for evidence of hatching, then held for later examination to determine whether the surviving larvae developed to maturity and produced a second generation. Larvae and pupae were examined for mortality following treatment, and survivors were held so that one could see whether the moths would mature and produce a second generation.

Each fumigant was tested through a range of concentrations beginning with the concentration that was expected to produce complete mortality of each life stage in a 24-hour exposure. The concentrations were reduced by one-half for each subsequent fumigation. The fumigations were conducted in pairs with a single set of control test insects for each pair of tests held in an adjacent untreated fumatorium (bottle). Concentration and exposure time combinations that resulted in survival of the test insects were replicated twice. Concentrations of the single and multiple component fumigants were determined by gas chromatographic analysis of air samples that were obtained by microsyringe after the test insects were inserted into the bottles. Concentrations in the fumatoria were adjusted between fumigations by dilution with outside air until analysis indicated the previous concentration had been reduced by about 50 percent. Additional moisture was added to the fumatoria as required to maintain a relative humidity of between 50 to 60 percent during each fumigation.

In an attempt to simulate the variable component ratios that often occur in EDC-CCl₄ fumigations, two component ratios were used in the EDC-CCl₄ fumigations (9), (7). One ratio consisted of three parts EDC to one part CCl₄, and the other ratio was one part EDC to one part CCl₄. The combined total (mg./l.) of the two components was about the same at each concentration level tested.

RESULTS

Chloropicrin Fumigations

Concentration-mortality data and observations on the development of test insects that survived fumigation with chloropicrin are in table 1. The order of tolerance to chloropicrin fumigation of the various life stages of the Indian meal moth was pupae > eggs > 5-week-old larvae > 2-week-old larvae.

Pupae began emerging as adults in the 4-hour exposures at concentrations of 7.8 mg./l., but failed to produce a second generation at concentrations as low as 2 mg./l. Survivors among 5-week-old larvae and larvae hatching from eggs matured and produced a second generation in 4-hour exposures at 2 mg./l.; however, none of the life stages survived 24-hour exposures at 2 mg./l. Pupae survived 24-hour exposures at 1.0 and 0.5 mg./l., but again were unable to produce a second generation. Eggs survived 24-hour exposures at 0.5 mg./l. and produced a second generation. None of the life stages survived 48-hour exposures at 0.5 mg./l. Exposures of 48 hours also gave complete control of both larval stages at the lowest concentration tested, 0.25 mg./l.

Table 1.—Mortality data obtained in the fumigation of eggs, larvae, and pupae of the Indian meal moth, Plodia interpunctella (Hübner), with chloropicrin

		Concentration	Mortality¹					
Concentration	Exposure time	time product	2-week old larvae	5-week-old larvae	Pupae	Egg hatch		
Milligrams per								
liter	Hours	Number	Percent	Percent	Percent	Yes/No		
4.0	4	56.0	100	100	100	No		
	24	336.0	100	100	100	No		
	48	672.0	100	100	100	No		
7.8	4	31.2	100	100	³ 70	No		
	24	187.2	100	100	100	No		
	48	374.4	100	100	100	No		
3.9	4	15.6	100	100	³ 50	No		
	24	93.6	100	100	100	No		
	48	187.2	100	100	100	No		
2.0	4	8.0	100	² 10	³ 20	² Yes		
•	24	48.0	100	100	100	No		
	48	96.0	100	100	100	No		
1.0	4	4.0	100	² 10	² 10	2 Yes		
	24	24.0	100	100	³ 50	No		
	48	48.0	100	100	100	No		
.5	4	2.0	² 40	² 10	² 0	2 Yes		
	24	12.0	100	100	320	$^2\mathrm{Yes}$		
	48	24.0	100	100	100	No		
.25	4	1.0	$^{2}20$	² 10	² 0	2 Yes		
	24	6.0	³ 80	³ 80	² 0	² Yes		
	48	12.0	100	100	³ 30	$^2\mathrm{Yes}$		

¹Mortality of untreated larvae and pupae did not exceed 10 percent for each set of control test insects. Each life stage of the untreated insects produced a second generation.

Phosphine Fumigations

Concentration-mortality data and observations on the development of test insects that survived fumigation with phosphine are given in table 2. The order of tolerance to phosphine fumigation was eggs > pupae > 5-week-old larvae > 2-week-old larvae. Eggs were particularly resistant to phosphine, surviving 24-hour exposures at 0.70 mg./l. (500 p.p.m.) and 72-hour exposures at 0.083 mg./l. (60 p.p.m.). Even with exposure of the eggs for 72 hours at 0.17 mg./l. (125 p.p.m.), the embryos were visible through the chorion and clearly formed. It was evident that death did not occur until near the final stage of embryonic development. Adults, that were only partially developed, emerged from pupae exposed for 24 hours at 0.35 mg./l. (250 p.p.m.), but quickly died. Adults emerging from pupae exposed for 24 hours at 0.17 mg./l. (125 p.p.m.) and for 48 hours at 0.021 mg./l. (15 p.p.m.) were fully developed, but failed to produce a second generation.

Mortality counts of the exposed 5-week-old larvae at preselected intervals were found unreliable in evaluating the effectiveness of phosphine fumigation. Fumigation in some instances resulted in partial paralysis, particularly in the posterior segments of the larvae; however, the larvae survived for as long as 25 days without further development. In other treatments, the anterior segments of the 5-week-old larvae showed signs of pupal formation, but the posterior segments retained larval characteristics until death. No second generation moths were produced from 2-week-old larvae surviving the lowest concentration (0.021 mg./l.) and shortest exposure time (4 hours) tested. Five-week-old larvae, however, were not affected by 4- or 24-hour exposures at 0.21 mg./l. (15 p.p.m.).

²Survivors matured to adult stage and produced a second generation.

³ Survivors matured to adult stage but did not produce a second generation.

Table 2.—Mortality data obtained in the fumigation of eggs, larvae, and pupae of the Indian meal moth, Plodia interpunctella (Hübner), with phosphine

		Concentration-	ationMortality¹					
Concentration	Exposure time	time product	2-week-old larvae	5-week-old larvae	Pupae	Egg hatch		
Milligrams per								
liter	Hours	Number	Percent	Percent	Percent	Yes/No		
0.70	4	2.8	100	100	² 50	· ² Yes		
(500 p.p.m.)	24	16.8	100	100	100	² Yes		
	48	33.6	100	100	100	No		
.35	4	1.4	100	² 10	² 10	² Yes		
(250 p.p.m.)	24	8.4	100	100	⁴ 100	² Yes		
	48	16.8	100	100	100	No		
	72	25.2	100	100	100	No		
.17	4	.68	100	² 10	² 30	² Yes		
(125 p.p.m.)	24	4.08	100	100	³ 90	² Yes		
	48	8.16	100	100	100	No		
	72	12.24	100	100	100	No		
.083	4	.33	100	² 10	20	² Yes		
(60 p.p.m.)	24	1.99	100	100	² 40	² Yes		
	48	3.98	100	100	100	² Yes		
	72	5.98	100	100	100	² Yes		
.042	4	.17	⁴ 100	^{3 4} 0	² 20	² Yes		
(30 p.p.m.)	24	1.01	100	^{3 4} 30	² 50	$^2\mathrm{Yes}$		
	48	2.02	100	⁴ 100	100	² Yes		
	72	3.02	100	⁴ 100	100	² Yes		
.021	4	.08	^{3 4} 70	² 0	² 10	² Yes		
(15 p.p.m.)	24	.50	100	² 0	² 30	² Yes		
	48	1.01	100	^{3 4} 90	³ 90	$^2\mathrm{Yes}$		
	72	1.51	100	⁴ 100	100	² Yes		

¹Mortality of untreated larvae and pupae did not exceed 10 percent for each set of control test insects. Each life stage of the untreated insects produced a second generation.

EDC-CCl4 Fumigations

Concentration-mortality data and observations on the development of test insects that survived fumigation with the three to one ratio of EDC-CCl $_4$ are in table 3. Data for the one to one ratio of EDC-GCl $_4$ are in table 4. The order of tolerance to both ratios of the EDC-CCl $_4$ was eggs > pupae > 5-week-old larvae > 2-week-old larvae. Exposures of 4 hours were not effective against the egg stage at total component concentrations of 50 mg./l.; however, the EDC-CCl $_4$ mixtures were effective against the pupal stages in 4-hour exposures of 12 mg./l. None of the life stages survived the 24- or 48-hour exposures in the three to one ratio fumigations within the range of concentrations (100 to 3 mg./l.) tested. Some difference in mortality was found between the two component ratios. Pupae survived 4-hour exposures at 6 mg./l. with each ratio, but those surviving exposure to the three to one ratio did not produce a second generation. Pupae exposed to the one to one ratio produced a second generation. Partial development in the pupae and eggs was observed in the 24-hour exposures at 3 mg./l. in the one to one ratio; however, both the adults emerging from the pupae and the larvae hatching from the eggs were abnormally formed and soon died.

² Survivors matured to adult stage and produced a second generation.

³ Survivors matured to adult stage but did not produce a second generation.

⁴One or more survivors partially developed to next life stage, then died.

Table 3.—Mortality data obtained in the fumigation of eggs, larvae, and pupae, of the Indian meal moth, *Plodia interpunctella* (Hübner), with three parts EDC to one part CCl₄ (75:25) mixture

Concentration					Mortality¹				
Total	Ethylene dichloride	Carbon tetra- chloride	Exposure time	Concentration- time product	2-week- old larvae	5-week- old larvae	Pupae	Egg hatch	
Milligrams per liter	Milligrams per liter	Milligrams per liter	Hours	Number	Percent	Percent	Percent	Yes/No	
100	75.0	25.0	4 24	100 2400	100 100	100 100	100 100	No No	
50	37.5	12.5	4 24 48	200 1200 2400	100 100 100	100 100 100	100 100 100	No No No	
22	18.5	5.5	4 24 48	88 528 1056	100 100 100	100 100 100	100 100 100	²Yes No No	
12	9.0	3.0	4 2 4 48	48 288 576	100 100 100	100 100 100	100 100 100	²Yes No No	
6	4.5	1.5	4 24 48	24 144 288	100 100 100	100 100 100	³ 50 100 100	²Yes No No	
3	2.25	.75	4 24 48	12 72 144	² 60 100 100	$^{2}50$ 100 100	² 10 100 100	² Yes No No	

¹Mortality of untreated larvae and pupae did not exceed 10 percent for each set of control test insects. Each life stage of the untreated insects produced a second generation.

CCl₄-CS₉ (80:20) Fumigations

Concentration-mortality data and observations on the development of test insects that survived fumigation with CCl₄-CS₂ are in table 5. The order of tolerance to CCl₄-CS₂ fumigation was eggs > pupae > 5-week-old larvae > 2-week-old larvae. The CCl₄-CS₂ mixture was the least effective of the four fumigants tested. Eggs, pupae, and 5-week-old larvae survived 4-hour exposures at 50 mg./l. and produced a second generation. Each life stage survived 24-hour exposures at 12.5 mg./l. and, with the exception of the 2-week-old larvae, produced a second generation.

Untreated Control Test Insects

Considerable experimentation was required in the collection and handling of the various life stages to develop methods which would not injure the insects nor disrupt their development. Cannibalism was prevalent among newly hatched larvae, and pupae were easily injured during collection. Diapause was observed among some 5-week-old larvae, perhaps induced by the density factors discussed by Tsuji (10). Hatching of the eggs began after 96 hours, which was earlier than the time period (50 percent hatch between 120-132 hours; first hatch at 108 hours) reported by Morrison and Crawford (5).

²Survivors matured to adult stage and produced a second generation.

³ Survivors matured to adult stage but did not produce a second generation.

Table 4.—Mortality data obtained in the fumigation of eggs, larvae, and pupae, of the Indian meal moth, *Plodia interpunctella* (Hübner), with one part EDC to one part CCl₄ (50:50 mixture)

Concentration					Mortality ¹				
Total	Ethylen <i>e</i> dichlorid <i>e</i>	Carbon tetra- chloride	Exposure time	Concentration- time product	2-week- old larvae	5-week- old larvae	Pupae	Egg hatch	
Milligrams per liter	Milligrams per liter	Milligrams per liter	Hours	Number	Percent	Percent	Percent	Yes/No	
100	50.0	50.0	4 24	400 2400	100 100	100 100	100 100	No No	
50	25.0	25.0	4 24 48	200 1200 2400	100 100 100	100 100 100	100 100 100	No No No	
24	12.0	12.0	4 24 48	96 576 1152	100 100 100	100 100 100	100 100 100	² Yes No No	
12	6.0	6.0	4 24 48	48 288 576	100 100 100 100	100 100 100 100	100 100 100 100	² Yes No No	
6	3.0	3.0	4 24 48	24 144 288	100 100 100	³ 100 100 100	² 30 100 100	² Yes No No	
3	1.5	1.5	4 24 48	12 72 144	² 20 100 100	² 10 100 100	² 0 ³ 100 100	² Yes ³ Yes No	

¹Mortality of untreated larvae and pupae did not exceed 10 percent for each set of control test insects. Each life stage of the untreated insects produced a second generation.

DISCUSSION

In evaluating the effectiveness of the fumigants tested in the study, concentration-time combinations that can readily be obtained in the top portion of stored grain had to be considered. Tests with chloropicrin used alone or mixed with CCl_4 - CS_2 in bulk stored grain, Storey (8), showed that chloropicrin remained in proportionately higher concentrations in the top 5 feet of grain than in deeper portions of the grain mass. During fumigation of a large, flat storage with chloropicrin applied by the recirculation method at the rate of 2 pounds per 1,000 cubic feet, concentrations at the $2\frac{1}{2}$ -foot depths exceeded the 2 mg./l. concentrations which gave complete control of all life stages in 24-hour exposures.

In field tests with EDC-CCl₄ (75:25) and CCl₄-CS₂ (80:20) in shelled corn stored in 3,250-bushel bins, Storey et al. (9), more insects survived in the upper part of the corn mass in the CCl₄-CS₂ (80:20) fumigations and in the lower part in the EDC-CCl₄ (75:25) fumigations. This was attributed to the EDC sorbing rapidly in the top portion of the corn and CS₂ penetrating more readily to the bottom levels. Concentrations obtained at the surface and 2½-foot levels in the field tests with EDC-CCl₄ (75:25) applied at the rate of 5 gallons per 1,000 bushels far exceeded the 3 mg./l. concentrations which produced complete mortality of all life stages in 24-hour exposures. In similar tests with CCl₄-CS₂ (80:20), total component concentrations of 50 mg./l. for 24 hours or 25 mg./l. for 48 hours was not obtained at all surface locations.

²Survivors matured to adult stage and produced a second generation.

³One or more survivors partially developed to next life stage, then died.

Table 5.—Mortality data obtained in the fumigation of eggs, larvae, and pupae, of the Indian meal moth, Plodia interpunctella (Hübner), with CCl₄-CS₂ (80:20 mixture)

Concentration			Mortality ¹					
Total	Carbon tetra- chloride	Carbon disulfide	Exposure time	•	2-week- old larvae	5-week- old larvae	Pupae	Egg hatch
Milligrams per liter	Milligrams per liter	Milligrams per liter	Hours	Number	Percent	Percent	Percent	Yes/No
50.0	40.0	10.0	4 24 48	200 1200 2400	100 100 100	² 60 100 100	² 20 100 100	² Yes No No
25.0	20.0	5.0	4 24 48	100 600 1200	³ 70 100 100	² 40 100 100	² 20 ³ 90 100	² Yes ² Yes No
12.5	10.0	2.5	4 24 48	50 300 600	² 50 ³ 70 100	² 10 ² 20 ² 50	² 0 ² 0 ³ 30	² Yes ² Yes ² Yes
5.5	4.4	1.1	4 24 48	22 132 264	² 40 ² 40 ² 60	² 0 ² 20 ² 20	² 0 ² 0 ² 10	² Yes ² Yes ² Yes
3.0	2.4	.6	72	216	³ 70	² 60	² 20	3Yes
1.5	1.2	.3	72	108	³ 70	² 10	² 20	² Yes

¹ Mortality of untreated larvae and pupae did not exceed 10 percent for each set of control test insects. Each life stage of the untreated insects produced a second generation.

A comparison of the concentration-time data in the phosphine fumigations show that high concentrations and short exposures were less effective than lower concentrations and longer exposure periods. Similar results were reported by Lindgren and Vincent (3) in phosphine fumigations of several species of stored-product insects. In addition, they found that eggs of the confused flour beetle, Tribolium confusum Jacquelin duVal, became less tolerant to phosphine with increasing age. This relationship becomes particularly important when phosphine fumigation is considered as a moth control treatment. Accumulations of substantial concentrations of phosphine in the surface area of grain is difficult to attain. McGregor (4) reported traces of phosphine in the surface area of corn in concrete silo-type storage during a 96-hour exposure following treatment at 2.3 tablets per ton. In treatments at 6.3 tablets per ton, however, concentrations reached 1.2 mg./l. after 72 hours. In limited field tests by Storey (unpublished data) 3,250-bushel bins of corn and soybeans were treated with 60 tablets (about ½ tablet per ton) placed just beneath the surface of the commodity. The absence of any substantial accumulation of phosphine within the top 2 feet of grain suggested that disposition of the phosphine from the surface of the grain occurred at nearly the same rate as evolution of the gas.

Data obtained in the study suggest that only a relatively small amount of phosphine would be required beyond the 4- to 6-day egg development period to kill the newly emerged larvae. Concentrations of 0.021 mg./l. (15 p.p.m.) for 24 hours gave complete control of the 2-week-old larvae. If concentrations of phosphine in this range could be sustained beyond hatching of the eggs, the problem of high tolerance of the egg stage to phosphine could be circumvented.

²Survivors matured to adult stage and produced a second generation.

³Survivors matured to adult stage but did not produce a second generation.

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